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(A)

Decreased Serum Tryptophan in Patients with HIV-1 Infection Correlates with Increased Serum Neopterin and with Neurologic/Psychiatric Symptoms

Dietmar Fuchs, *Arnulf A. Möller, Gilbert Reibnegger, †Elisabeth Stöckle, Ernst R. Werner, and Helmut Wachter

*Institute of Medical Chemistry and Biochemistry and Ludwig Boltzmann Institute of AIDS Research, University of Innsbruck, Innsbruck, Austria, *Max Planck Institute of Psychiatry, Clinical Institute, Munich, and †4th Department of Internal Medicine, Clinic Munich/Schwabing, Munich, F.R.G.*

Summary: We investigated serum neopterin, tryptophan, and kynurenine concentrations in 23 HIV-1 seropositive patients (Walter Reed Stage 4–6). Ten patients presented with polyneuropathy and three with dementia, one of the patients with dementia also had polyneuropathy and dementia. We found significant associations between lower tryptophan concentrations and neurologic/psychiatric symptoms. The negative correlation of tryptophan with kynurenine and neopterin concentrations indicates activity of indoleamine 2,3-dioxygenase (IDO) in patients. IDO can be induced by cytokines such as interferon-γ and therefore low tryptophan levels may result from chronic immune stimulation in HIV-1 seropositives. **Key Words:** Serum tryptophan—Neopterin—Dementia—Polyneuropathy.

Neurologic dysfunction and destruction are frequent complications of human immunodeficiency virus type 1 (HIV-1) infection. However, the mechanisms by which symptoms such as dementia or polyneuropathy are mediated have not been elucidated (1). Disturbance of tryptophan metabolism was suggested to be involved (2) and reduced tryptophan together with increased kynurenine as well as reduced serotonin concentrations were reported to occur frequently in the sera of patients with advanced HIV-1 infection (3,4). We were interested in investigating a possible association between disturbed tryptophan metabolism, chronic immune stimulation, and neurologic/psychiatric symptoms in patients with HIV-1 infection.

Address correspondence and reprint requests to Prof. Dr. H. Wachter, Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Fritz Pregl Str. 3. A-6020 Innsbruck, Austria.

We investigated serum tryptophan, kynurenine, and neopterin concentrations by high-performance liquid chromatography (3) in 23 patients (all male, 12 homosexuals, 6 intravenous drug abusers, 5 homosexuals with intravenous drug abuse; median age 35.0 years, range 22–54) with proven HIV-1 infection (Abbott enzyme-linked immunosorbent assay positive, confirmed by Western blot). Four patients were classified as Walter Reed Stage (WR) 4, 9 were WR5, and 10 were WR6. Ten patients (three with WR4, three with WR5, four with WR6) presented with polyneuropathy. One of the WR6 patients with polyneuropathy also had dementia as did two further WR6 patients without polyneuropathy.

Polyneuropathy was diagnosed clinically and confirmed by electroneurophysiological examination, which was performed in each case of patient complaints (cramps, dysesthesia) or findings (disturbances of motor and/or sensory functions afflicting the extremities, loss of tendon reflexes) sugges-

EXHIBIT B

(3)

tive of affection of peripheral nerves. To test for neurologic dysfunction and destruction we quantified ventricular enlargement as measured by the ventricle–brain ratio in computerized cranial tomography (5).

The score of a structured interview and brief neuropsychological testing was used for the diagnosis of dementia. The tests rely on DSM-III-R and ICD-10 algorithms including the Mini Mental State (6) and allow a detailed measurement of even low levels of cognitive impairment. The test provides quantification of severity grading of cognitive dysfunctions that are reflected by lower scores (highest score is 55).

Statistical comparisons were performed applying Student's t test. For correlation analyses, Spearman's rank correlation coefficients (r_s) were used.

Serum tryptophan levels were lower in our patients with HIV-1 infection compared with healthy blood donors (Table 1), which confirms earlier results (3). Moreover, patients with polyneuropathy and/or dementia had lower serum tryptophan levels compared with those without such symptoms (Table 1). Three patients with dementia had the lowest tryptophan levels (31.2, 34.4, and 34.8 μmol/L). In contrast, serum kynurenine and neopterin levels were higher in patients compared with healthy controls. Neopterin and kynurenine levels did not differ in patients with additional symptoms compared with those without (Table 1).

Tryptophan and neopterin levels were negatively correlated ($r_s = -0.608$, $p < 0.01$). In addition, tryptophan and neopterin concentrations were correlated with ventricle brain ratio (neopterin: $r_s = 0.588$, $p < 0.01$; tryptophan: $r_s = -0.444$, $p < 0.05$) as well as with the dementia score (neopterin: $r_s = -0.461$, $p < 0.05$; tryptophan: $r_s = 0.447$, $p < 0.05$).

The significant correlations with neopterin may indicate that chronic immune activation is involved in the disturbed tryptophan metabolism and the pathogenesis of neurologic/psychiatric symptoms. Large amounts of neopterin are produced by human monocytes/macrophages on stimulation with interferon-γ (7), and other cytokines such as tumor necrosis factor α can amplify its action (8). Recently, interferon-γ and tumor necrosis factor α were reported to be increased in sera of patients with HIV-1 infection (9,10). In vitro data showed that interferon-γ is also able to induce indoleamine 2,3-dioxygenase (IDO), which degrades tryptophan to kynurenine (8). In our patients with HIV-1 infection, increased activity of IDO is indicated by increased serum kynurenine levels in patients with decreased tryptophan. A positive correlation existed between kynurenine and neopterin levels ($r_s = 0.419$, $p < 0.05$), which supports the concept that chronic immune activation and increased degradation of tryptophan, rather than reduced dietary intake, may be the reason for reduced tryptophan levels. The independence from the nutritional status is further supported by the finding that serum albumin did not differ between patients with and without dementia and/or polyneuropathy. In addition, no correlation between serum albumin and any other variable of the study was found (data not shown).

Kynurenine levels were high in HIV-1 seropositive patients but no further increase was seen in the patients with dementia and/or polyneuropathy compared with those without. This finding agrees with in vitro observations that IDO is the only enzyme within the metabolic pathway of tryptophan degradation that is activated by interferon-γ (8). Other enzymes downstream in the degradation pathway are constitutively present in cells. Thus, kynurenine formed by IDO is likely to be further metabolized

TABLE 1. *Tryptophan, kynurenine, and neopterin levels (mean ± SD) in HIV-1 seropositive patients with and without dementia and/or polyneuropathy (for comparison levels on HIV-1 seronegative blood donors are shown)*

	Tryptophan (μmol/L)		Kynurenine (μmol/L)		Neopterin (nmol/L)	
HIV-1 seropositive patients with dementia and/or polyneuropathy (n = 12)	48.8 ± 13.4	t = 3.59, p < 0.01	3.5 ± 0.78	t = 0.25, NS[a]	30.8 ± 19.8	t = 1.90, NS[a]
HIV-1 seropositive patients without dementia and/or polyneuropathy (n = 11)	70.5 ± 15.6	t = 2.53, p < 0.05	3.6 ± 1.14	t = 3.13, p < 0.01	18.6 ± 8.2	t = 5.61, p < 0.01
HIV-1 seronegative blood donors (n = 11)	91.1 ± 22.0		2.3 ± 0.77		4.5 ± 1.5	

[a] Student's t test; NS, not significant.

downstream in the degradation pathway of tryptophan to form 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and quinolinic acid. Quinolinic acid has already been demonstrated to be increased in patients with ARC and AIDS (11).

Activation of IDO can also be represented by the ratio of the product concentration (kynurenine) versus the substrate concentration (tryptophan). A highly significant positive correlation between the kynurenine/tryptophan quotient and neopterin (Fig. 1) indicates that both events, neopterin release and activation of IDO, can be referred to the specific activity of cytokines, such as interferon-γ and tumor necrosis factor α.

Our data show that low serum tryptophan is associated with neurologic dysfunction in HIV-1 infection. Decreased serum tryptophan as well as increased neopterin are associated with the degree of neurologic disturbances in patients with HIV-1 infection as expressed by correlations between the markers and computed tomography results as well as dementia scores. Reduced tryptophan levels are very likely to result from persistent immune activation. This is underlined by the strong negative correlation between neopterin and tryptophan concentrations. Moreover, the best correlation was found between neopterin levels and the ratio of kynurenine to tryptophan, representing an estimate of IDO activity, an enzyme that is inducable by cytokines.

In summary, all biochemical changes discussed may result from continuous release of cytokines such as interferon-γ and tumor necrosis factor α.

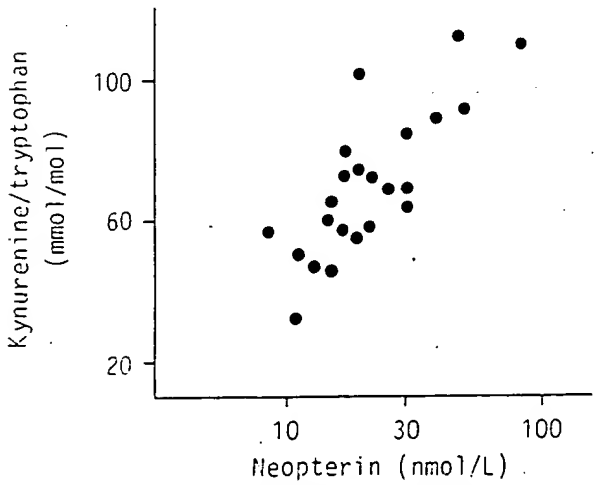

FIG. 1. Correlation between serum neopterin levels and the ratio between kynurenine and tryptophan concentrations as an estimate for indoleamine (2,3)-dioxygenase (r_s = 0.826, $p < 0.01$; Spearman rank correlation coefficient).

The background of chronic immune activation may be multifactorial. Besides HIV-1, other infectious agents could be important. However, highly increased neopterin concentrations were not only found in HIV-seropositive individuals in high-risk groups but also in transfusion recipients (12).

Increased neopterin concentrations can be observed early during the course of HIV-1 infection (7,13), and neopterin levels further increase during the course of the infection. However, only chronic and high-level immune activation appears to cause degradation of tryptophan in a range that is relevant compared with the dietary intake.

Low tryptophan concentrations may explain decreased serum serotonin levels that were reported to occur frequently among HIV-1 seropositive patients (4). We expect that similar associations between active tryptophan metabolism and chronic immune activation can also be established intrathecally. Reduced tryptophan and serotonin levels (14) and increased neopterin levels (13,15) in the cerebrospinal fluid have already been shown to be associated with dementia and/or polyneuropathy in patients with HIV-1 infection.

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